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Observations on the life history and field biology of an imperiled butterfly *Philotiella leona* (Lepidoptera: Lycaenidae) from South Central Oregon

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Abstract. Observations on the life history and field biology of the highly range-restricted south-central Oregon butterfly, *Philotiella leona* Hammond and McCorkle (Lepidoptera: Lycaenidae), are presented. Oviposition was difficult to obtain in captivity. Eggs were laid on or adjacent to flower buds of the larval host Spurry buckwheat, *Eriogonum spergulinum* (Polygonaceae) and hatch in 4-5 days at 25-27°C. Larvae feed only on flower buds and flowers, have four instars and reach the pre-pupal stage after 10-12 days at 25-27°C. Pupae are formed after a further 5-6 days and overwinter. Adults fly from mid June to the end of July, males eclosing a few days before females. Mating and oviposition occur soon after eclosion and the sex ratio is relatively balanced through most of the flight period. Flight is meandering and low to the ground, most common after mid morning when sunny and temperatures are > 21 °C. Nectaring occurs on at least six species of flowering plants.

Keywords: *Philotiella leona*, endangered species, life history, biology, *Eriogonum spergulinum*

Introduction

Leona's little blue butterfly, *Philotiella leona* Hammond and McCorkle, is arguably the most range-restricted and endangered butterfly species in the United States. Discovered in 1995, *P. leona* is restricted to less than 32 km² in the Antelope Desert of south central Oregon (Hammond & McCorkle, 2000; Pyle, 2002; Warren, 2005; Miller & Hammond, 2007; Ross, 2008; 2009; Matheson *et al.*, 2010). It appears to be a highly specialized species occupying a volcanic ash and pumice ecosystem using the desert-restricted annual Spurry buckwheat, *Eriogonum spergulinum* A. Gray (Polygonaceae) as its larval host. *Eriogonum spergulinum* occurs commonly in similar desert habitats in California, Nevada and Idaho (Goodman, 1948). *Philotiella leona* is currently being considered for listing under the Endangered Species Act (Matheson *et al.*, 2010). Apart from brief and fragmentary notes presented by Hammond & McCorkle (2000), Ross (2008, 2009) and Matheson *et al.* (2010), little is known of the biology or life history of *P. leona*. This paper provides images and information on the development of immature stages of *P. leona* as well as field observations on adult biology.

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Materials and methods

Immature stages. *Philotiella leona* was reared in the laboratory from gravid females, eggs and larvae collected from the Antelope Desert in Klamath County, Oregon during June-July 2011. On July 1, seven females showing oviposition behavior were obtained, stored in plastic containers in a cool box (10-15°C) and transported to Prosser, WA. A second group of 20 females was obtained on July 7 and a third group (n = 8) on July 25. Seven eggs and one fourth instar larva were collected on July 16. Flowering stems of *E. spergulinum* were collected randomly from one (~ 0.5 ha) location on July 24 and 29 and stored in three plastic containers (34 × 24 × 12 cm) on each date. Containers were held at 25-27 °C and inspected daily for wandering *P. leona* larvae. Larvae were collected, measured (to identify instar) and reared (see below). Gravid females were held individually or in groups of 4-12 in small, cylindrical plastic containers (12 × 13 or 23 × 13 cm) with gauze lids for oviposition. Host plants (*E. spergulinum*) with flower buds (July 1) or flowers (July 7, 25) were provided in containers as cut stems in water or as potted plants. Nectar was provided by *E. spergulinum* flowers and supplemented with cut flowers of *Achillea millefolium* L. Paper towel pads soaked in dilute sucrose solution were provided for additional nourishment. Most oviposition cages were held indoors at 25-30°C under natural day lengths and exposed to sunshine for at least 8 hours/day. Some cages were held under fluorescent lighting (natural day lengths) at 35-39°C (day) and 25°C (night). When females died they were frozen for later dissection to determine ovarian and mating status. Larvae were reared individually in glass tubes with gauze lids or communally (up to 20 individuals) in a plastic container (16 × 28 × 7 cm) with a gauze lid. A small stem of *E. spergulinum* bearing buds and flowers with the cut end in a small ball of moist cotton wool was provided for each larva in glass tubes and replaced every other day. Multiple stems with buds and flowers were provided to larvae in the plastic container and replaced after 3-4 days. Larvae were reared at 25-30°C under natural day lengths from July 17-August 15. Individually reared eggs and larvae were used to determine egg and instar sizes (using a microscope eyepiece graticule) and durations. Observations were made of larvae feeding, molting and pupating. High quality images were taken of eggs, all instars and pupae using a Canon digital SLR camera (EOS 1DS Mark II) mounted on a tripod. A Canon MP-E 65mm 1X – 5X macro lens was used together with a Macro Twin Lite MT – 24 EX flash lighting system.

Adult biology. Field observations on flight, sex ratio, nectaring, roosting, mating and oviposition behavior of *P. leona* adults were made during walks (12-15 hrs) through multiple patches of *E. spergulinum* during each of six weekly visits to the Antelope Desert population from June 21-July 29 2011.

RESULTS

Observations during rearing in captivity

Oviposition. Oviposition by *P. leona* in captivity was poor. A total of only 12 eggs was obtained, 7 of these within 3 days from females obtained on July 1 and held individually with cut *E. spergulinum* at flower bud stage. Three eggs were laid on potted *E. spergulinum* by a group of 5 females held at 25-39°C under fluorescent lighting. The majority of females did not lay eggs, despite having well developed ovaries, as indicated by dissection of individuals that died. A mean of 39.3 ± 12.9 (SE) mature eggs were present in ovaries of six females that died during July 11-12, compared to 12.6 ± 2.1 in six females that died during July 24-26 ($P = 0.015$, Mann-Whitney rank sum test, SigmaStat V 3.0). Eggs were mostly placed on or at the base of flower buds (Figs. 1-2). Two eggs were placed on a stem ~ 5 cm distant from buds.

Egg. The egg measures 0.48 ± 0.01 mm (n =12) in width and is pale whitish-yellow (Fig. 1). The micropyle is relatively wide and contained within a slight depression. The surface is covered with a network of obscure ridges. Hatching occurs after 4-5 days at 25-27°C, taking ~ 1 hour with the larva eating a jagged hole through the top of the egg shell, exiting without consuming it (Fig. 2).

First instar. After hatching the first instar measures 1.0 ± 0.2 (SE) mm (n = 10). It is dull creamy-yellow, clothed with moderate length white setae arising from dark spots. The head is shiny brown (Fig. 2). After 24-36 hours of feeding, a green tinge develops. Light orange-red markings appear after 2 days forming a dorsal stripe and two indistinct lateral stripes. There is also a sub-spiracular pale stripe. Prior to molting the larva is suffused with increased reddish pigmentation and measures 2.0 ± 0.2 mm in length (n = 8) (Fig. 1).

Second instar. After molting to second instar the larva measures 2.0 ± 0.2 mm in length (n = 8) and is reddish with indistinct white markings which form two stripes laterally, the lower one better defined than the upper one. A darker red mid-dorsal stripe is present and the head is shiny black in this and all subsequent instars. Sparse, short white setae cover the body and the larva increases in length to 4.0 ± 0.1 mm (n = 8) (Fig. 1).

Third instar. The early third instar measures 4.0 ± 0.1mm (n = 8) and is bright red with distinct white blotches dorsally and a prominent, wide sub-spiracular white stripe. The mid dorsal dark red stripe is broken into prominent spots, one per segment. The white setae are shorter and denser and the larva increases in length to 6.0 ± 2.0 mm (n = 8) (Figs. 1-2).

Fourth instar. The final instar develops from 6.0 ± 2.0 mm (n = 8) to 10.0 ± 3.0 mm (n = 10) and is mostly white with vivid blood-red markings, resulting from an interrupted mid-dorsal stripe and two lateral stripes either side of the sub-spiracular white stripe. Short white setae densely cover the body. Ventrally, the larva is red with yellow claspers and black true legs (Fig. 1).

Pre-pupa. When the larva reaches 7.0-10.0 mm in length, the dorsal white markings become suffused with red and it shrinks to 5.0-7.0 mm. After ~ 24 hours the larva becomes a sessile and uniformly yellow colored pre-pupa (Fig. 1). A single strand of silk may girdle the pre-pupa but this was seen in only 4 of 12 pupae examined.

Pupa. The pupa is uniformly orange-brown with darker wing cases and an indistinct mid-dorsal stripe on the abdomen. The spiracles are dark brown (Fig. 1). Pupa length ranges from 4.0-7.0 mm (mean 5.5 ± 0.25 (SE) (n = 11)). Pupation occurred mostly on the ground (n =9) but three pupae were formed on host plant stems.

Larval development and behavior. Development from egg hatch to pre-pupa was rapid at 25-27°C occupying 10-12 days. Larval development may be even faster in the field as judged by the relative proportions of instars in samples of larvae obtained on July 24 and 29. During these 5 days the larval population changed from early instar (1 & 2) dominated (73%, n = 26) to late instar (3 & 4) dominated (92%, n = 25). All instars fed only on the unopened flower buds and flowers of *E. spergulinum*. The petals were eaten together with other flower parts including perhaps pollen (Fig. 2). If flowers were not available all early instar (1-3) larvae died. Two final instar larvae attempted early pupation while only measuring 6-7mm in length. Both produced deformed, non-viable pupae. The pre-pupal stage is relatively prolonged occupying 5-6 days. This is unusual in northwest US lycaenids (James and Nunnallee 2011). In captivity, most pre-pupae were formed at the bottom of the cage and pre-pupal wandering was not observed. The pupal period lasts for at least 10-11 months but some pupae may remain dormant for more than a year. Dave McCorkle (pers. comm.) reports that some *P. leona* pupae remain dormant for 2 years in captivity before eclosing. From approximately 45-50 larvae reared all were the red color form described above. Matheson *et al.* (2010) reported the rare occurrence of a green larval form. Field observations in 2011 suggest the incidence of green form larvae to be ~ 1 in 100 (Gary Pearson, pers. comm.).

Observations on adult biology

The flight period occupied 6-7 weeks, from about June 18 to July 31. Males emerged a few days before females, producing an early male-skewed sex ratio. From early July until the end of the flight period the sex ratio was relatively balanced (Table 1). Spontaneous flight behavior did not generally occur until mid-late morning when air temperatures were > 21°C. Cloudy and/or windy conditions inhibited flight. Flight is low (usually < 0.3 m above the ground) and meandering with little or no straight line flight. Males search for females among patches of host plant and mating occurs soon after female eclosion. During late June-early July mating pairs were frequently (5-10 pairs/day) seen resting on *E. spergulinum* and other low-growing plants, usually during late afternoon. Oviposition occured from late June to the end of the flight period, peaking in early July (Table 1). Eggs were laid (mostly singly but occasionally two) only on budding *E. spergulinum* (25 observations). Nectaring was observed on many low growing flowering plants but was most common on *E. spergulinum*, *Gayophytum diffusum* Torr. & A. Gray (Onagraceae), *Attenaria rosea* greene (Asteraceae), *Plagiobothrys hispidus* A. Gray (Boraginaceae), *Hemizonella minima* A. Gray (Asteraceae) and later in the flight period *Eriogonum umbellatum* Torr. (Polygonaceae). Males were observed to visit up to 67 flowers of *H. minima* in 12 minutes. Both sexes spent much time roosting or resting on dead twigs, dried grass blades and seed heads just above the ground. During morning hours, resting individuals oriented themselves so that ventral wing surfaces face the sun.

DISCUSSION

This study provides the first detailed observations on the life history and adult biology of *P. leona*. Rearing was relatively easy, however, obtaining oviposition from females was difficult. Best success was obtained with separately-caged individuals supplied on July 1 with budding *E. spergulinum*. Field observations indicated that *P. leona* oviposits only on budding *E. spergulinum* and the confinement of females with flowering rather than budding host plants on July 7 and 25 may have suppressed oviposition. Timing of larval feeding is critical, with flowers of *E. spergulinum* providing the apparent sole source of nourishment. Larvae of *Philotiella speciosa* also feed only on flowers when using the buckwheat, *Eriogonum reniforme* Torr. and Frem. (closely related to *E. spergulinum*) (Scott, 1986). Flower and seed feeding on *Eriogonum* spp. is also widespread in *Euphilotes* spp. (Pratt, 1994; Peterson, 1997). The immature stages of *P. leona* show typical lycaenid characters particularly those found in the related genus *Euphilotes*. The egg appears to be smooth but has fine undulating, irregular surface ridges. The micropyle-containing depression on the top of the egg appears to be unique among lycaenid species in the Pacific Northwest (James and Nunnallee 2011). The vibrant and contrasting red and white marked third and fourth instar larvae are cryptic on the reddish host plant and bear some resemblance to the last instar larvae of *Euphilotes columbiae* (Mattoni) and *Euphilotes enoptes* (Boisduval) (James and Nunnallee 2011). The apparent fourth instars of *P. leona* shown

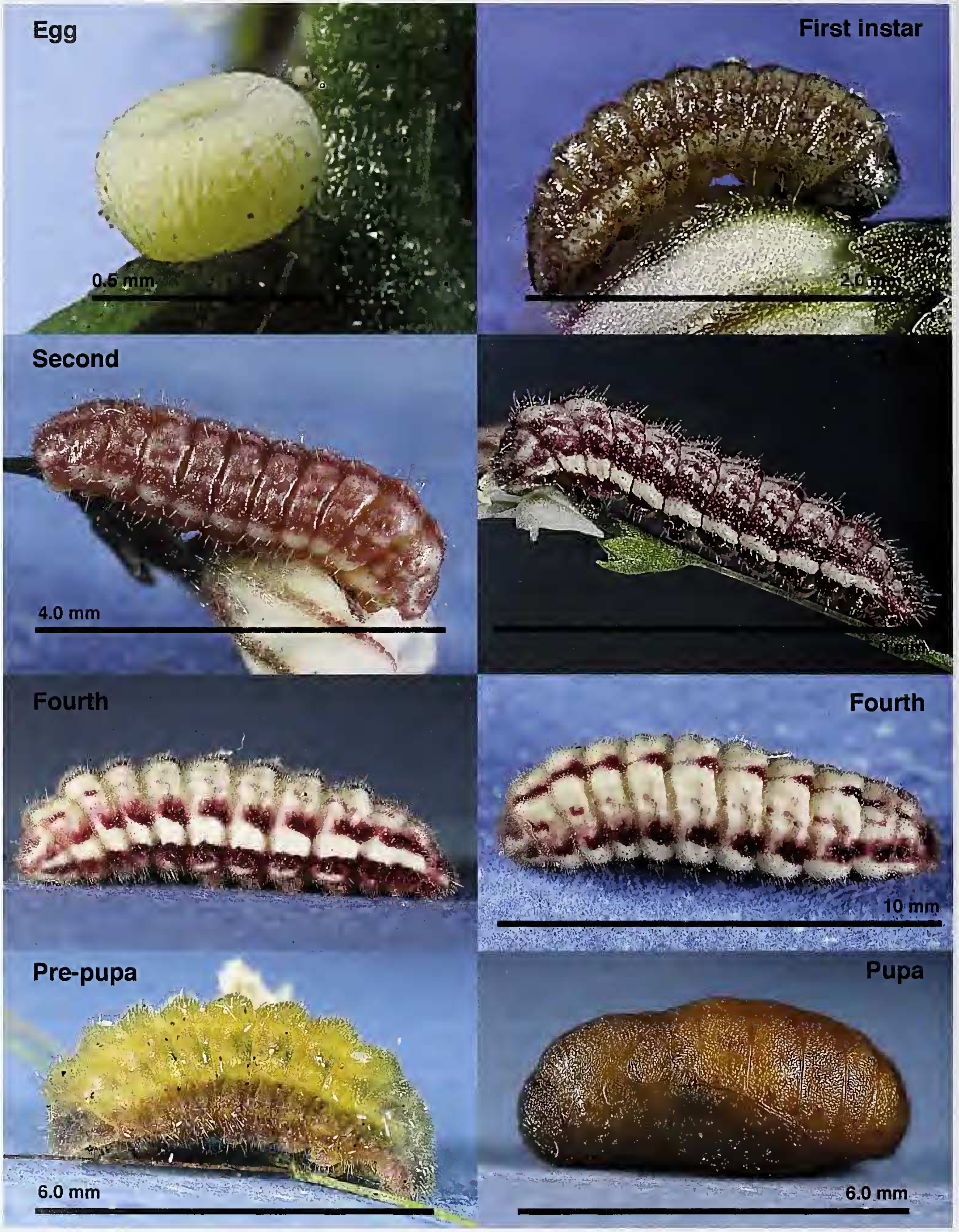

Figure 1. Life cycle stages of *P. leona*.

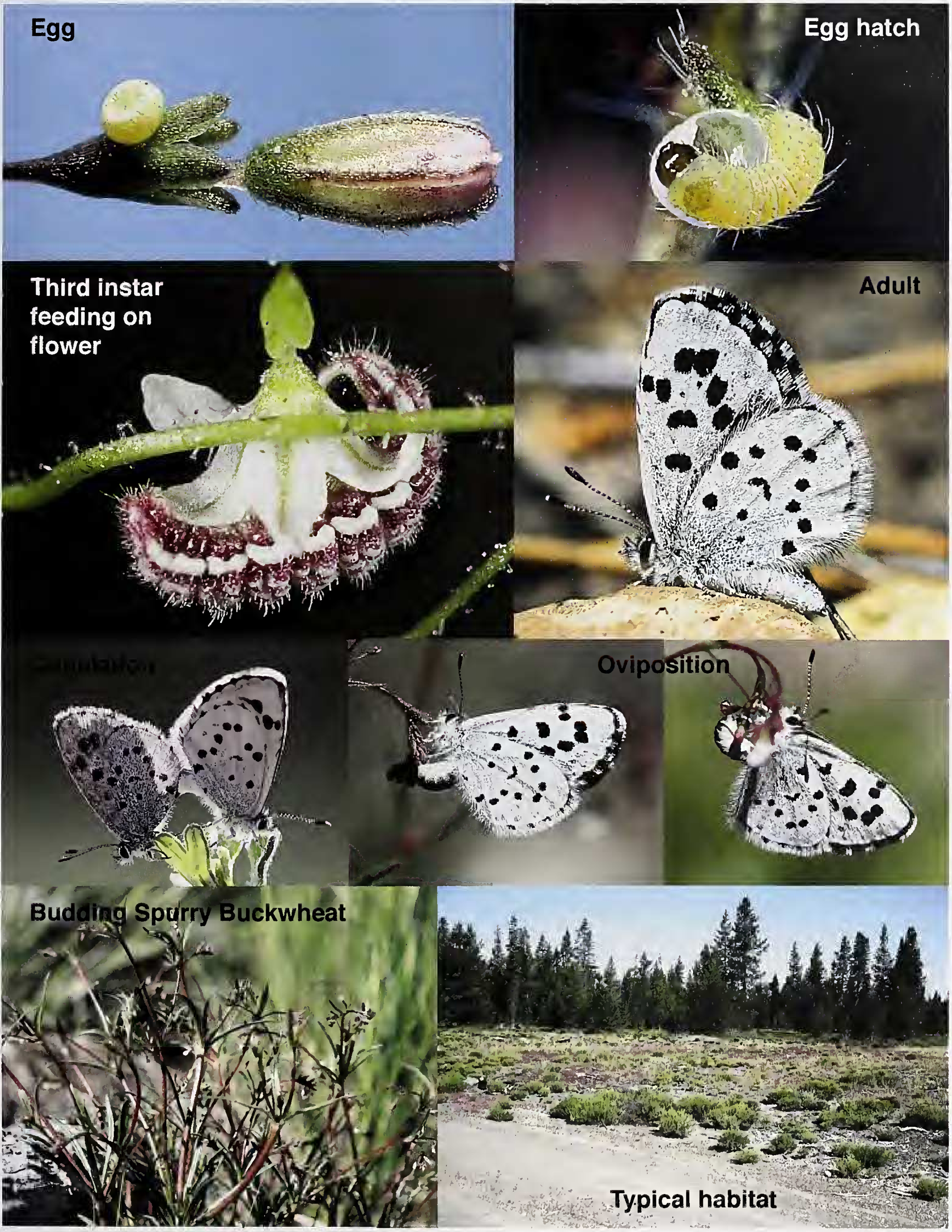

Figure 2. Aspects of the biology and ecology of *P. leona*

Table 1. Sex ratio (%) of *P. leona* adults captured or observed during June 21-July 28 2011. Bottom line shows number of oviposition events observed at each visit.

Sex	June 21-22	June 30-July 1	July 7-9	July 15-16	July 22-23	July 28-29
Male (%)	100	64.7	58.1	41.3	69.4	40
Female (%)	0	35.3	41.9	58.7	30.6	60
Total (n)	33	184	148	63	36	5
Oviposition events (n)	0	3	15	9	0	0

in Ross (2008, 2009) and Matheson *et al.* (2010) are identical to ours. The larva shown in Miller & Hammond (2007) is greener but has a similar mid-dorsal dark red stripe. The apparent low incidence of green form larvae is unsurprising given their conspicuousness on the predominantly red host plant. Color polymorphism is common in Lycaenidae larvae and often related to host plant color variation (Ballmer & Pratt, 1988; James & Nunnallee, 2011). Mature larvae of *P. speciosa* may be similar but in Allen *et al.* (2005) the larva of *P. speciosa* is dull red and lacks strongly contrasting white markings. Ballmer & Pratt (1988) described the ground color of *P. speciosa* larvae as 'green or yellowish'.

The observations presented here provide the basis for further laboratory and field studies on the biology and ecology of *P. leona*. In particular information is needed on the impact of weather and natural enemies on survival of immature stages and adults. The likelihood of extended pupal dormancy also needs investigating. Prolonged pupal diapause has been reported in some *Euphilotes* spp. (Austin *et al.*, 2008; Pratt & Ballmer, 1987) and in *Glaucopsyche piasus* (Boisduval) (James & Nunnallee, 2011). It has also been reported for the closely related *P. speciosa* (Monroe & Monroe, 2004; Allen *et al.*, 2005) and appears to occur frequently in desert butterflies generally (Sands & New, 2008; James & Nunnallee, 2011). Critically, we need data on the population dynamics of *P. leona* over several years together with information on environmental variables, to assess whether the population is in decline, increasing or stable. A comprehensive set of data on the biology and ecology of *P. leona* will enable the development of land management strategies that permit the best chance of survival for this charismatic but severely restricted species.

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